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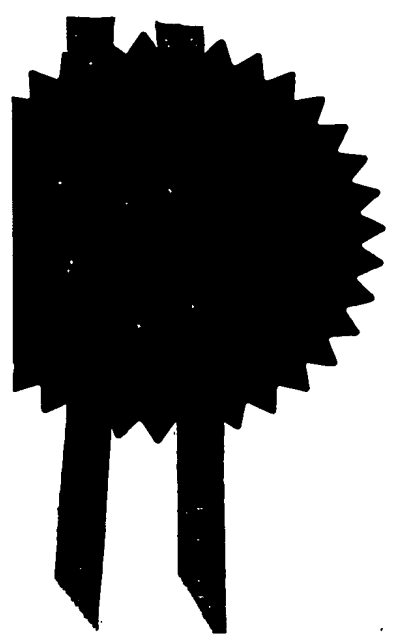
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1. Your reference

AWGP/JW/PG4790

2. Patent application number

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28 MAR 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

Glaxo Group Limited
Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN, Great Britain

United Kingdom

473587 003

4. Title of the invention

Novel Compounds

5. Name of your agent (*if you have one*)

"Address for service" in the United Kingdom to which all correspondence should be sent

(including the postcode)

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Corporate Intellectual Property

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- b) there is an inventor who is named as an applicant, or
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Patents Form 1/77

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11.

We request the grant of a patent on the basis of this application

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H B Dawson

Date 28-Mar-02

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Patents Form 1/77

Novel Compound

This invention relates to a novel compound, a process for its preparation, pharmaceutical formulations containing it and its use in therapy.

5 Inflammation is a primary response to tissue injury or microbial invasion and is characterised by leukocyte adhesion to the endothelium, diapedesis and activation within the tissue. Leukocyte activation can result in the generation of toxic oxygen species (such as superoxide anion), and the release of granule products (such as peroxidases and proteases). Circulating leukocytes include
10 neutrophils, eosinophils, basophils, monocytes and lymphocytes. Different forms of inflammation involve different types of infiltrating leukocytes, the particular profile being regulated by the profile of adhesion molecule, cytokine and chemotactic factor expression within the tissue.

The primary function of leukocytes is to defend the host from invading
15 organisms, such as bacteria and parasites. Once a tissue is injured or infected, a series of events occurs which causes the local recruitment of leukocytes from the circulation into the affected tissue. Leukocyte recruitment is controlled to allow for the orderly destruction and phagocytosis of foreign or dead cells, followed by tissue repair and resolution of the inflammatory infiltrate. However in
20 chronic inflammatory states, recruitment is often inappropriate, resolution is not adequately controlled and the inflammatory reaction causes tissue destruction.

There is increasing evidence that the bronchial inflammation which is characteristic of asthma represents a specialised form of cell-mediated immunity, in which cytokine products, such as IL-4 and IL-5 released by T-helper 2 (Th2)
25 lymphocytes, orchestrate the accumulation and activation of granulocytes, in particular eosinophils and to a lesser extent basophils. Through the release of cytotoxic basic proteins, pro-inflammatory mediators and oxygen radicals, eosinophils generate mucosal damage and initiate mechanisms that underlie bronchial hyperreactivity. Therefore, blocking the recruitment and activation of
30 Th2 cells and eosinophils is likely to have anti-inflammatory properties in asthma. In addition, eosinophils have been implicated in other disease types such as rhinitis, eczema, irritable bowel syndrome and parasitic infections.

Chemokines are a large family of small proteins which are involved in trafficking and recruitment of leukocytes (for review see Luster, New Eng. J.
35 Med., 338, 436-445 (1998)). They are released by a wide variety of cells and act to attract and activate various cell types, including eosinophils, basophils, neutrophils, macrophages, T and B lymphocytes. There are two major families of chemokines, CXC- (α) and CC- (β) chemokines, classified according to the spacing of two conserved cysteine residues near to the amino terminus of the
40 chemokine proteins. Chemokines bind to specific cell surface receptors

belonging to the family of G-protein-coupled seven transmembrane-domain proteins (for review see Luster, 1998). Activation of chemokine receptors results in, amongst other responses, an increase in intracellular calcium, changes in cell shape, increased expression of cellular adhesion molecules, degranulation and
 5 promotion of cell migration (chemotaxis).

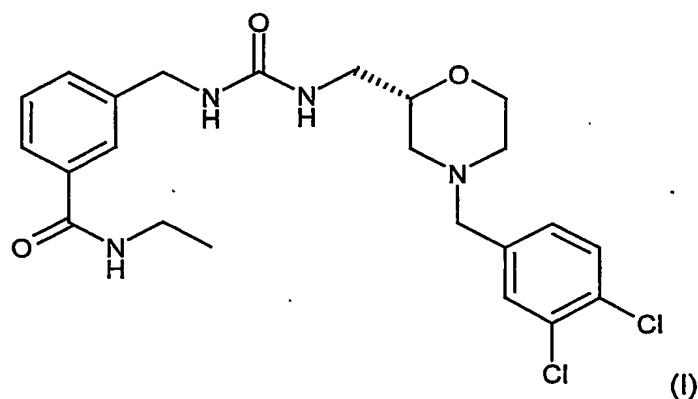
To date a number of CC chemokine receptors have been identified and of particular importance to the current invention is the CC-chemokine receptor-3 (CCR-3), which is predominantly expressed on eosinophils, and also on
 10 basophils, mast cells and Th2 cells. Chemokines that act at CCR-3, such as RANTES, MCP-3 and MCP-4, are known to recruit and activate eosinophils. Of particular interest are eotaxin and eotaxin-2, which specifically bind to CCR-3. The localization and function of CCR-3 chemokines indicate that they play a central role in the development of allergic diseases such as asthma. Thus, CCR-3 is specifically expressed on all the major cell types involved in inflammatory
 15 allergic responses. Chemokines that act at CCR-3 are generated in response to inflammatory stimuli and act to recruit these cell types to sites of inflammation, where they cause their activation (e.g. Griffiths et al., J. Exp. Med., 179, 881-887 (1994), Lloyd et al., J. Exp. Med., 191, 265-273 (2000)). In addition, anti-CCR-3 monoclonal antibodies completely inhibit eotaxin interaction with eosinophils
 20 (Heath, H. *et al.*, J. Clin. Invest. 99 (2), 178-184 (1997)), while an antibody for the CCR-3 specific chemokine, eotaxin, reduced both bronchial hyperreactivity and lung eosinophilia in an animal model of asthma (Gonzalo et al., J. Exp. Med., 188, 157-167 (1998). Thus, many lines of evidence indicate that antagonists at the CCR-3 receptor are very likely to be of therapeutic use for the treatment of a
 25 range of inflammatory conditions.

In addition to a key role in inflammatory disorders, chemokines and their receptors also play a role in infectious disease. Mammalian cytomegaloviruses, herpes viruses and pox viruses express chemokine receptor homologues, which can be activated by human CC chemokines such as RANTES and MCP-3
 30 receptors (for review see Wells and Schwartz, Curr. Opin. Biotech., 8, 741-748, 1997). In addition, human chemokine receptors, such as CXCR-4, CCR-5 and CCR-3, can act as co-receptors for the infection of mammalian cells by microbes such as human immunodeficiency viruses (HIV). Thus, chemokine receptor antagonists, including CCR-3 antagonists, may be useful in blocking infection of
 35 CCR-3 expressing cells by HIV or in preventing the manipulation of immune cellular responses by viruses such as cytomegaloviruses.

International Patent Application publication number WO 01/24786 (Shionogi & Co. Ltd.) discloses certain aryl and heteroaryl derivatives for treating diabetes. WO 00/69830 (Torrey Pines Institute for Molecular Studies) discloses
 40 certain diazacyclic compounds, and libraries containing them, for biological

- screening. WO 00/18767 (Neurogen Corporation) discloses certain piperazine derivatives as dopamine D4 receptor antagonists. United States Patent 6,031,097 and WO 99/21848 (Neurogen Corporation) discloses certain aminoisoquinoline derivatives as dopamine receptor ligands. WO 99/06384
- 5 (Recordati Industria Chimica) discloses piperazine derivatives useful for the treatment of neuromuscular dysfunction of the lower urinary tract. WO 98/56771 (Schering Aktiengesellschaft) discloses certain piperazine derivatives as anti-inflammatory agents. WO 97/47601 (Yoshitomi Pharmaceutical Industries Ltd.) discloses certain fused heterocyclic compounds as dopamine D-receptor
- 10 blocking agents. WO 96/39386 (Schering Corporation) discloses certain piperidine derivatives as neurokinin antagonists. WO 96/02534 (Byk Gulden Lomberg Chemische Fabrik GmbH) discloses certain piperazine thiopyridines useful for controlling helicobacter bacteria. WO 95/32196 (Merck Sharp & Dohme Limited) discloses certain piperazine, piperidine, and tetrahydropyridine
- 15 derivatives as 5-HT1D-alpha antagonists. United States Patent 5,389,635 (E.I. Du Pont de Nemours and Company) discloses certain substituted imadazoles as angiotensin-II antagonists. European Patent Application publication number 0 306 440 (Schering Aktiengesellschaft) discloses certain imidazole derivatives as cardiovascular agents.
- 20 A novel compound has now been found which is a CCR-3 antagonist. This compound blocks the migration/chemotaxis of eosinophils and thus possesses anti-inflammatory properties. This compound is therefore of potential therapeutic benefit, especially in providing protection from eosinophil, basophil mast cell and Th2-cell-induced tissue damage in diseases where such cell types
- 25 are implicated, particularly allergic diseases, including but not limited to bronchial asthma, allergic rhinitis and atopic dermatitis.

Thus, according to one aspect of the invention, there is provided a compound of formula (I):



which is;

3-((((((2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl)methyl)amino)carbonyl]-
-amino)methyl)-N-ethylbenzamide;

and salts and solvates thereof.

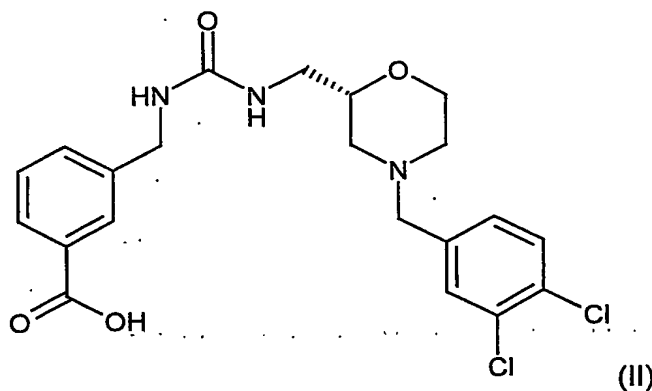
- 5 Suitable salts of the compound of formula (I) include physiologically acceptable salts and salts which may not be physiologically acceptable but may be useful in the preparation of compounds of formula (I) and physiologically acceptable salts thereof. If appropriate, acid addition salts may be derived from inorganic or organic acids, for example hydrochlorides, hydrobromides,
- 10 sulphates, phosphates, acetates, benzoates, citrates, succinates, lactates, tartrates, fumarates, maleates, 1-hydroxy-2-naphthoates, pamoates, methanesulphonates, formates or trifluoroacetates.

Examples of solvates include hydrates.

- 15 The compound of formula (I) and salts and solvates thereof may be prepared by the methodology described hereinafter, constituting a further aspect of this invention.

A process according to the invention for the preparation of the compound of formula (I) comprises coupling of a compound of formula (II) or a salt thereof with ethylamine:

20



and thereafter, if required, carrying out one or more of the following optional steps:

- 25 (i) removing any protecting group(s);
 (ii) preparing an appropriate salt or solvate of the compound so formed.

- The coupling of the compound of formula (II) is carried out in any suitable solvent, for example a polar organic solvent such as a mixture of tetrahydrofuran and N,N-dimethylformamide in the presence of a suitable dehydrating agent such as a carbodiimide reagent e.g. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
- 30 hydrochloride, and optionally a suitable activating agent, e.g. an activated

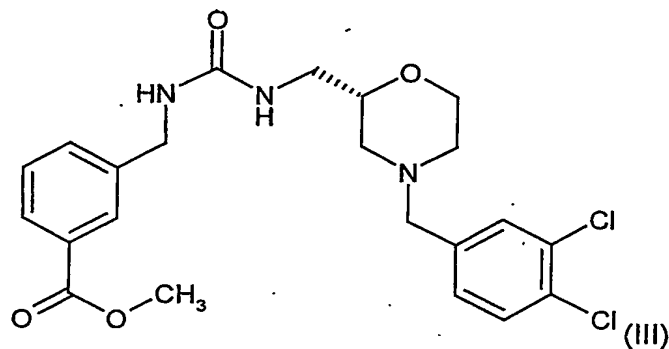
hydroxy compound such as 1-hydroxybenzotriazole, and optionally in the presence of a base such as a tertiary amine, e.g. N,N-diisopropylethylamine, under conventional coupling conditions at any temperature providing a suitable rate of formation of the required product, over a suitable reaction time, for

5 example 12 - 24 hours.

Suitable reaction temperatures include those in the range of 18°C to 25°C.

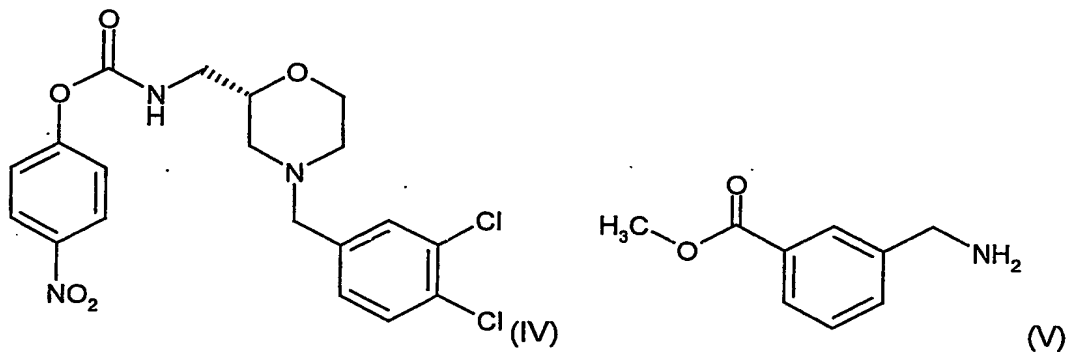
The reaction products are isolated and purified using conventional methods.

10 The compound of formula (II) may be prepared by hydrolysis of the compound of formula (III):



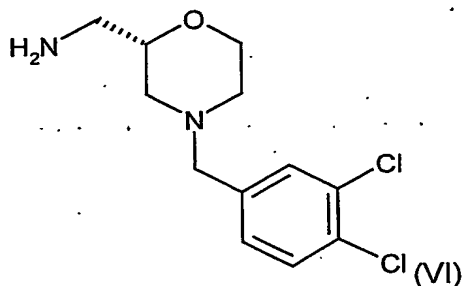
15 The hydrolysis is carried out in a suitable solvent, for example an aqueous alcohol such as a mixture of water and methanol, in the presence of a suitable catalyst, for example an aqueous base such as sodium hydroxide, at a suitable temperature, for example those in the range of 18°C to 25°C over a suitable period of time, for example 12 - 24 hours.

20 The compound of formula (III) may be prepared by reaction of the compound of formula (IV) with the compound of formula (V):



The reaction between the compound of formula (IV) and the compound of formula (V) is performed in a suitable solvent, such as a polar organic solvent, for example N,N-dimethylformamide and a suitable base, for example a tertiary amine such as N,N-diisopropylethylamine, at a suitable temperature, for example those in the range of 18°C to 25°C over a suitable period of time, for example 60 - 100 hours.

The compound of formula (IV) may be prepared from the compound of formula (VI)

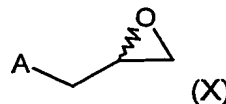
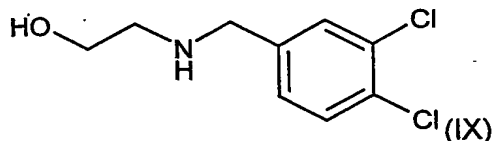


by reaction with 4-nitrophenyl chloroformate.

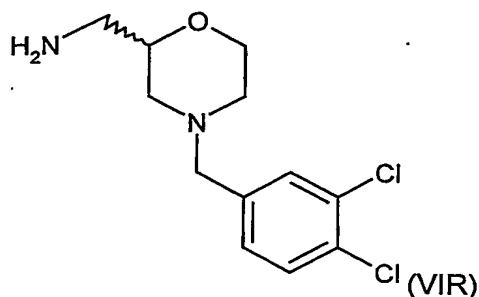
The reaction between the compound of formula (VI) and 4-nitrophenyl chloroformate is performed in a suitable solvent, for example an inert organic solvent such as dichloromethane, in the presence of a suitable base, for example a tertiary amine such as triethylamine, at a suitable temperature, for example those in the range of -5°C to +5°C over a suitable period of time, for example 3-5 hours.

A compound of formula (VI) may be prepared by either by Reaction (a), Reaction (b), or Reaction (c).

Reaction (a). Reaction of the compound of formula (IX) with a compound of formula (X)



wherein A is a protected amino group, suitably phthalimido, followed by deprotection of the amino group to give a compound of formula (VIR)



followed by resolution of the resulting enantiomers of the compound of formula (VIR);

5 or;

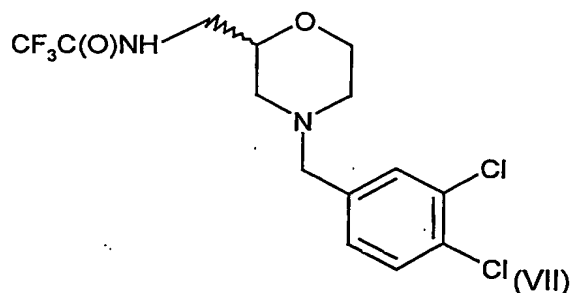
Reaction (b). Reaction of a compound of formula (IX) as hereinbefore defined with a compound of formula (XA)



10

wherein A is as hereinbefore defined for formula (X), followed by deprotection of the amino group to give the compound of formula (VI).

Reaction (c). Hydrolysis of the compound of formula (VII);



15

followed by resolution of the resulting enantiomers of a compound of formula (VIR).

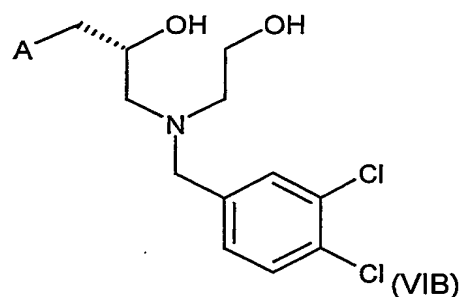
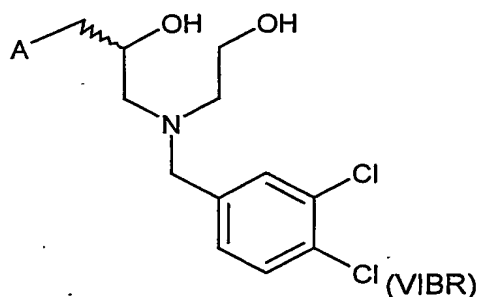
20 For both reactions (a) and (b), the cyclisation of the intermediate diols (VIBR) and (VIB) in the reaction between the compound of formula (IX) and a compound of formula (X) or (XA) is typically carried out under the Mitsunobu conditions as follows:

25 Typically, a mixture of the compound of formula (IX) and the compound of formula (X) or formula (XA) in a suitable solvent, such as tetrahydrofuran, is stirred, suitably for 20-24 hours at a suitable temperature, suitably the reflux temperature of the solvent, under an inert atmosphere, suitably an atmosphere of

nitrogen. Further solvent is then added and the mixture cooled, suitably to 0-5°C. A suitable phosphine, suitably triphenyl phosphine, is added and the mixture stirred until all the solid is dissolved. A suitable azo compound, suitably diisopropylazodicarboxylate, is then added over a period of time, suitably, 10-15 minutes, while maintaining the temperature at <7°C. The mixture is allowed to stand for a period of time, suitably 2-3 hours, then allowed to warm, suitably to 20-25°C. After a further period of standing, suitably 4-6 hours, further phosphine and azo compound are added. After a further period of standing, suitably 20-24 hours, the reaction mixture is concentrated to near dryness. A suitable alcohol, suitably propan-2-ol, is added and the concentration step repeated; the alcohol addition and concentration step is then repeated. Further alcohol is then added and the mixture heated to a temperature suitably between 65-75°C. After a suitable period, suitably 20-45 minutes, the resultant slurry is cooled, suitably to 20-25°C, and then allowed to stand, suitably for 1.5 – 3 hours, after which time the product is isolated by filtration. The filter bed is washed with more alcohol and then dried *in vacuo* at 35-45°C to yield the protected form of the compound of formula (VIR) or formula (VI) respectively.

The removal of the protecting group from the product is typically carried out as follows. A slurry of the protected form of the compound of formula (VIR) or formula (VI) in an appropriate polar solvent, suitably water, is heated to elevated temperature, suitably 70-75°C and then treated dropwise with a concentrated mineral acid, suitably concentrated sulphuric acid. The mixture was then heated at elevated temperature, suitably the reflux temperature of the solvent, for a suitable period of time, suitably 20-24 hours, after which the reaction mixture was cooled to 20-25°C and then treated with a suitable apolar solvent, suitably dichloromethane. A base, suitably 0.880 ammonia solution, is then added dropwise, maintaining the temperature between 20-25°C. Further apolar solvent is then added, the aqueous phase then being separated and extracted with further apolar solvent. The combined organic phase is washed with water and then evaporated to dryness. The residue is redissolved and the apolar solvent evaporated to give the compound of formula (VIR) or formula (VI).

The process for the preparation of the protected form of the compound of formula (VIR) or formula (VI) described above may also be undertaken in two stages, in which an intermediate compound of formula (VIBR) or of formula (VIB) respectively;



wherein A is as hereinbefore defined for formulae (X) and (XA);
is isolated.

- 5 Typically, a mixture of the compound of formula (IX) and a compound of formula (X) or formula (XA) in a suitable solvent, such as tetrahydrofuran, is stirred, suitably for 20-24 hours at a suitable temperature, suitably the reflux temperature of the solvent, under an inert atmosphere, suitably an atmosphere of nitrogen. Further compound of formula (IX) is added and the mixture heated at a
- 10 suitable temperature, suitably the reflux temperature of the solvent, under an inert atmosphere, suitably an atmosphere of nitrogen, for a suitable period of time, suitably 3-6 hours. The reaction mixture is then cooled, suitably to 20-25°C, and the compound precipitated by means of addition of a suitable co-solvent, suitably diisopropyl ether. The compound of formula (VIBR) or formula
- 15 (VIB) respectively is isolated by filtration, washed with further co-solvent and dried *in vacuo*.

A protected form of the compound of formula (VIR) or formula (VI) may then be prepared from a compound of formula (VIBR) or formula (VIB) under similar conditions to those of the reaction between a compound of formula (IX) and formulae (X) or (XA) as hereinbefore described, but omitting the reflux period

20 prior to the addition of the phosphine and azo compounds.

Reaction (c) is typically carried out by stirring a solution of the compound of formula (VII) in a suitable solvent, for example a mixture of methanol and water, and adding a suitable base, for example potassium carbonate. The

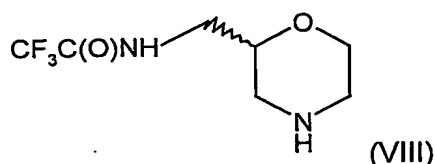
25 mixture is stirred at a suitable temperature, for example those in the range 20-25°C for a suitable time, for example 16-20 hours followed by removal of the organic solvent in vacuo. Water is then added and the mixture extracted with a suitable organic solvent, for example ethyl acetate. The combined organic phases are washed with water and saturated aqueous sodium chloride solution

30 before drying over a suitable drying agent, for example sodium sulphate, filtering and evaporation of the solvent in vacuo. The crude product is then purified by flash chromatography.

The resolution of the compound of formula (VI) from the racemic product i.e. the compound of formula (VIR) may be undertaken using techniques well

known to those skilled in the art, for example preparative chiral high performance liquid chromatography (chiral HPLC) or by fractional crystallisation of diastereoisomeric salts.

The compound of formula (VII) may be prepared by reaction of the
5 compound of formula (VIII)



with 3,4-dichlorobenzyl chloride.

10 The reaction between the compound of formula (VIII) and 3,4-dichlorobenzyl chloride is typically carried out in a suitable solvent, for example N,N-dimethylformamide, under an inert atmosphere, for example an atmosphere of nitrogen, with the addition of a suitable base, for example potassium carbonate, and a suitable activating agent, such as sodium iodide. The mixture
15 is then stirred at a suitable temperature, for example a temperature in the range of 20-25°C, for a suitable period of time, for example 16-20 hours before removing the volatile components in vacuo.

The compound of formula (VIII) is prepared by treating a solution of morpholin-2-ylmethylamine in a suitable organic solvent, for example methanol,
20 under an inert atmosphere, for example an atmosphere nitrogen, with a solution of ethyl- α,α,α -trifluoroacetate in a suitable organic solvent, for example ether. The mixture is then stirred for a suitable period of time, for example 20-40 minutes at a suitable temperature, for example a temperature in the range of 20-25°C and the volatile components removed in vacuo. The residue is then
25 dissolved in a suitable organic solvent, for example methanol, and the volatile components removed in vacuo.

The compound of formula (V) is known and may be prepared by conventional means, for example those disclosed in *Eur. J. Med. Chem.* (1993), 28(7-8), 625-32. Ethylamine, 4-nitrophenyl chloroformate, 2-[(3,4-
30 dichlorobenzyl)amino]ethanol, 2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione, 3,4-dichlorobenzyl chloride, morpholin-2-ylmethylamine, 2-[(3,4-dichlorobenzyl)amino]ethanol and ethyl- α,α,α -trifluoroacetate are known, commercially available compounds or may be prepared by analogy with known procedures, for example those disclosed in standard reference texts of synthetic
35 methodology such as *J. March, Advanced Organic Chemistry, 3rd Edition (1985), Wiley Interscience*.

The compounds of formulae (II), (III), (IV), (VI), (VII) (VIBR) and (VIB) are considered to be novel.

Accordingly, there is provided a compound of formula (II).

There is also provided a compound of formula (III).

5 There is also provided a compound of formula (IV).

There is also provided a compound of formula (VI).

There is also provided a compound of formula (VII).

There is also provided a compound of formula (VIBR).

There is also provided a compound of formula (VIB).

10 Suitable protecting groups in any of the above mentioned reactions are those used conventionally in the art. The methods of formation and removal of such protecting groups are those conventional methods appropriate to the molecule being protected, for example those methods discussed in standard reference texts of synthetic methodology such as *P J Kocienski, Protecting*
15 *Groups, (1994), Thieme.*

For any of the hereinbefore described reactions or processes, conventional methods of heating and cooling may be employed, for example electric hotplates and ice/salt baths respectively. Conventional methods of purification, for example crystallisation and column chromatography may be used
20 as required.

The salts and solvates of the compounds of formula (I) may be prepared from the compound of formula (I) or suitable salts or solvates thereof and isolated according to conventional procedures.

The compounds of the invention may be tested for *in vitro* biological
25 activity in accordance with the following assays:

(a) CCR-3 Binding Assay

A CCR-3 competition binding SPA (scintillation proximity assay) was used to assess the affinity of novel compounds for CCR-3. Membranes prepared
30 from K562 cells stably expressing CCR-3 (2.5µg/well) were mixed with 0.25mg/well wheat-germ agglutinin SPA beads (Amersham) and incubated in binding buffer (HEPES 50 mM, CaCl₂ 1 mM, MgCl₂ 5 mM, 0.5% BSA) at 4°C for 1.5 hr. Following incubation, 20 pM of [¹²⁵I] eotaxin (Amersham) and increasing concentrations of compound (1pM to 30µM) were added and incubated in a 96
35 well plate for 2 hr at 22°C then counted on a Microbeta plate counter. The total assay volume was 100 µl. Competition binding data were analysed by fitting the data with a four parameter logistic equation. Data are presented as the mean pIC₅₀ values (negative logarithm of the concentration of compound which inhibits [¹²⁵I]eotaxin binding by 50%) from at least two experiments.

40

(b) Eosinophil Chemotaxis Assay.

- The compound was evaluated for its inhibitory effect on eosinophil chemotaxis. Eosinophils were purified from human peripheral blood by standard CD16 cell depletion using a Miltenyi cell separation column and a magnetic Super Macs magnet as previously described (Motegi & Kita, 1998; J.Immunology. 161:4340-6). Cells were re-suspended in RPMI 1640/10% FCS solution and incubated with calcein-AM (Molecular Probes) at 37°C for 30 mins. Following incubation, the eosinophils were centrifuged at 400g for 5 min and re-suspended in RPMI/FCS at 2.2 million/ml. Cells were then incubated in the presence of increasing concentration of compounds (1 pM to 30 µM) at 37°C for 30 mins. For control responses cells were incubated with RPMI/FCS only. The agonist eotaxin (an EC₈₀ concentration) was added to the lower chamber of a 96 well chemotaxis plate (5 µm filter: Receptor Technologies). Eosinophils (50 µl of 2 million/ml cells) were added to the top chamber of the filter plate and incubated at 37°C for 45 mins. Cells remaining on top of the chemotaxis filter were removed and the number of eosinophils which had migrated were quantified by reading the plate on a fluorescent plate reader. Inhibition curves for the effect of compounds on eosinophil chemotaxis were analysed by fitting the data with a four parameter logistic equation. Functional pK_i values (fpK_i) were generated using the equation below (Lazareno & Birdsall, 1995. Br.J.Pharmacol 109: 1110-9).

$$fpKi = \frac{IC_{50}}{1 + \frac{[Agonist]}{EC_{50}}}$$

- The compound of the invention was tested in the CCR-3 binding and/or eosinophil chemotaxis assays (assays (a) and (b)). The compound of the invention was tested in the CCR-3 binding assay and possessed a pIC₅₀ value of 9.2. The compound of the invention tested in the CCR-3 eosinophil chemotaxis assay possessed an fpK_i value of 10.0.

- Examples of disease states in which the compound of the invention has potentially beneficial anti-inflammatory effects include diseases of the respiratory tract such as bronchitis (including chronic bronchitis), bronchiectasis, asthma (including allergen-induced asthmatic reactions), chronic obstructive pulmonary disease (COPD), cystic fibrosis, sinusitis and rhinitis.

- Also included are diseases of the gastrointestinal tract such as intestinal inflammatory diseases including inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis) and intestinal inflammatory diseases secondary to radiation exposure or allergen exposure.

Furthermore, the compound of the invention may be used to treat nephritis; skin diseases such as psoriasis, eczema, allergic dermatitis and hypersensitivity reactions; and diseases of the central nervous system which have an inflammatory component (eg. Alzheimer's disease, meningitis, multiple sclerosis), HIV and AIDS dementia.

The compounds of the present invention may also be of use in the treatment of nasal polyposis, conjunctivitis or pruritis.

Further examples of disease states in which the compound of the invention has potentially beneficial effects include cardiovascular conditions such as atherosclerosis, peripheral vascular disease and idiopathic hypereosinophilic syndrome.

The compound of the invention may be useful as an immunosuppressive agents and so has use in the treatment of auto-immune diseases such as allograft tissue rejection after transplantation, rheumatoid arthritis and diabetes.

The compound of the invention may also be useful in inhibiting metastasis.

Diseases of principal interest include asthma, COPD and inflammatory diseases of the upper respiratory tract involving seasonal and perennial rhinitis.

It will be appreciated by those skilled in the art that references herein to treatment or therapy extend to prophylaxis as well as the treatment of established conditions.

As mentioned above, the compound of formula (I) is useful as a therapeutic agent.

There is thus provided as a further aspect of the invention a compound of formula (I) or a physiologically acceptable salt or solvate thereof for use as an active therapeutic agent.

There is also therefore provided the compound of formula (I), or a physiologically acceptable salt or solvate thereof, for use in the treatment of inflammatory conditions, eg. asthma or rhinitis.

According to another aspect of the invention, there is provided the use of the compound of formula (I) or a physiologically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of inflammatory conditions, eg. asthma or rhinitis.

In a further or alternative aspect there is provided a method for the treatment of a human or animal subject suffering from or susceptible to an inflammatory condition eg. asthma or rhinitis, which method comprises administering an effective amount of the compound of formula (I) or a physiologically acceptable salt or solvate thereof.

The compound according to the invention may be formulated for administration in any convenient way.

There is thus further provided a pharmaceutical composition comprising the compound of formula (I), or a physiologically acceptable salt or solvate thereof, and optionally one or more physiologically acceptable diluents or carriers.

- 5 There is also provided a process for preparing such a pharmaceutical formulation which comprises admixing the compound of formula (I) or a physiologically acceptable salt or solvate thereof with one or more physiologically acceptable diluents or carriers.

- 10 The compound according to the invention may, for example, be formulated for oral, inhaled, intranasal, buccal, parenteral or rectal administration, preferably for oral administration.

- Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch, cellulose or polyvinyl pyrrolidone; fillers, for
 15 example, lactose, microcrystalline cellulose, sugar, maize- starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycollate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well
 20 known in the art.

- Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending
 25 agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl
 30 alcohol; or preservatives, for example, methyl or propyl *p*- hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

- 35 The compound may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

- The compound according to the invention may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume
 40 infusions or pre-filled syringes, or in multidose containers with an added

preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or tonicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.

The compound and pharmaceutical compositions according to the invention may also be used in combination with other therapeutic agents, for example antihistaminic agents, anticholinergic agents, anti-inflammatory agents such as corticosteroids, e.g. fluticasone propionate, beclomethasone dipropionate, mometasone furoate, triamcinolone acetonide or budesonide; or non-steroidal anti-inflammatory drugs (NSAIDs) eg. sodium cromoglycate, nedocromil sodium, PDE-4 inhibitors, leukotriene antagonists, iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine 2a agonists; or beta adrenergic agents such as salmeterol, salbutamol, formoterol, fenoterol or terbutaline and salts thereof; or antiinfective agents e.g. antibiotic agents and antiviral agents. It will be appreciated that when the compound of the present invention is administered in combination with other therapeutic agents normally administered by the inhaled or intranasal route, that the resultant pharmaceutical composition may be administered by the inhaled or intranasal route.

The compounds of the invention may conveniently be administered in amounts of, for example, 0.001 to 500mg/kg body weight, preferably 0.01 to 500mg/kg body weight, more preferably 0.01 to 100mg/kg body weight, and at any appropriate frequency e.g. 1 to 4 times daily. The precise dosing regimen will of course depend on factors such as the therapeutic indication, the age and condition of the patient, and the particular route of administration chosen.

Throughout the description and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

General experimental details

LC/MS System

This system used an 3 μ m ABZ+PLUS (3.3cm x 4.6mm internal diameter) column, eluting with solvents: A – 0.1%v/v formic acid + 0.077% w/v ammonium acetate in water; and B – 95:5 acetonitrile:water + 0.05%v/v formic acid, at a flow rate of 3 ml per minute. The following gradient protocol was used: 100% A for 0.7mins; A+B mixtures, gradient profile 0 – 100% B over 3.5mins; hold at 100%B for 1.1mins; return to 100% A over 0.2mins.

Thermospray Mass Spectra

Thermospray Mass Spectra were determined on a HP 5989A engine mass spectrometer, +ve thermospray, source temperature 250°C, probe temperatures 120°C (stem), 190°C (tip), detection mass range 100-850 a.m.u. Compounds were injected in 10 μ l of a mixture of solvents comprising 65% methanol and 35% 0.05M aqueous ammonium acetate, at a flow rate of 0.7ml/min.

Solid phase extraction (ion exchange)

'SCX' refers to Isolute Flash SCX-2 sulphonic acid solid phase extraction cartridges.
All temperatures are in °C

Descriptions

Description 1: 2,2,2-Trifluoro-N-(morpholin-2-ylmethyl)acetamide

To a stirred solution of morpholin-2-ylmethylamine (3.1g) in methanol (70ml) under nitrogen was added an ethereal solution of ethyl- α,α,α -trifluoroacetate (5ml in 20ml ether) which had been washed with saturated aqueous sodium bicarbonate, water and brine, and dried. The mixture was stirred for 30 min at 22°C before removal of all volatiles in vacuo. The residue was dissolved in methanol (10ml) and the volatiles again removed in vacuo to give the title compound as a white crunchy foam (4.9g).
Thermospray Mass Spectrum m/z 213 [MH⁺].

Description 2: N-[4-(3,4-Dichlorobenzyl)morpholin-2-yl]methyl]-2,2,2-

trifluoroacetamide

To a stirred solution of Description 1 (3.3g) in N,N-dimethylformamide (50ml) under nitrogen was added potassium carbonate (2.46g) and sodium iodide (2.12g). A solution of 3,4-dichlorobenzyl chloride (2ml) in N,N-dimethylformamide (10ml) was added dropwise to the mixture. The mixture was stirred at 22°C for 18h before the volatiles were removed in vacuo. The residue was partitioned between dichloromethane (100ml) and saturated aqueous sodium carbonate solution (50ml). The organic phase was subsequently washed with additional saturated aqueous sodium carbonate solution (2 x 50ml) and water (50ml) before drying over magnesium sulphate, filtering and evaporation of the solvent in vacuo to give a pale yellow oil. The oil was purified by Biotage

flash chromatography on a 90g silica cartridge eluting with 25% ethyl acetate in cyclohexane, to give the title compound as a colourless oil (2.97g).

LC/MS (System A) R_t 2.63 min, Mass Spectrum m/z 371 [MH^+].

5 Description 3: [4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine

To a stirred solution of Description 2 (2.97g) in methanol (15ml) and water (5ml) was added potassium carbonate (5.53g). The mixture was stirred at 22°C for 18h before the methanol was removed in vacuo. Water (25ml) was added and the mixture extracted with ethyl acetate (3 x 30ml). The combined organic

- 10 phases were washed with water (5ml) and saturated aqueous sodium chloride solution (10ml) before drying over sodium sulphate, filtering and evaporation of the solvent in vacuo to give a pale yellow oil. The oil was purified by Biotage flash chromatography on a 90g silica cartridge eluting with 75:8:1 dichloromethane/ethanol/0.880 ammonia solution. The required fractions were
- 15 combined and the solvent evaporated in vacuo to give the title compound as a colourless oil (1.85g).

LC/MS (System A) R_t 1.77 min, Mass Spectrum m/z 275 [MH^+].

20 Description 4: [4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine (alternative synthesis)

A mixture of 2-[(3,4-dichlorobenzyl)amino]ethanol (Chem Abs No. 40172-06-3, 0.980g) and 2-(oxiran-2-ylmethyl)-1H-indole-1,3(2H)-dione (1.10g) was heated at 80°C under nitrogen for 3h. The resulting solid mass was treated with concentrated sulphuric acid (1.5ml) then stirred at 150°C for 24h. The mixture

25 was treated with water (100ml) then washed with ethyl acetate (2x100ml). The dark aqueous phase was basified to ~pH 12 using 5M aqueous sodium hydroxide, then extracted with ethyl acetate (2x100ml). The combined organic extracts were washed with water and brine, dried (Na_2SO_4) and concentrated under vacuum to give the title compound as a brown oil (1.02g).

- 30 LC-MS (System A): R_t 1.6min.

Description 5: 1-[(2S)-4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine

Description 3 (racemic mixture, 8g) was separated into its single enantiomers by preparative chiral-HPLC. The separation was carried out using a 2" x 22cm

- 35 Chiralpak AD 20 μ m column, Merck self pack DAC system, eluting with 95:5:0.1 (v/v) heptane : absolute ethanol: diethylamine (flow rate: 55ml/min over 40min, UV detection 225nm); sample load preparation: 400mg sample in 20ml 3:2 (v/v) absolute ethanol: system eluent.

- 40 The title compound (2.49g) was obtained as follows: preparative HPLC retention time 23.0 min.

Description 5 (Alternative procedure)

- A slurry of Description 7 (1.00g) in water (8.5ml) was heated to 75° and then treated dropwise with concentrated sulphuric acid (2.5ml). The mixture was then
- 5 heated at reflux. After 23h the reaction mixture was cooled to 22° and then treated with dichloromethane (6ml). 880 Ammonia solution (7ml) was then added dropwise with cooling. More dichloromethane (10ml) was added. The aqueous phase was separated and extracted with more dichloromethane (10ml). The combined organic phase was washed with water (5ml) and then evaporated
- 10 to dryness. The residue was redissolved in dichloromethane and the solvent re-evaporated to give the product as an oil (662mg).

Description 6: 1-[(2S)-4-(3,4-Dichlorobenzyl)morpholin-2-yl]methanamine salt with D-tartaric acid 1:1

- 15 Description 3 (0.613g) was dissolved in methanol (12.3ml). D-Tartaric acid (0.335g) was added and the slurry was heated to reflux for 50min. The mixture was allowed to cool to 0-5°C and the precipitate isolated by filtration to give the title compound as a white solid (0.4g).
- ee: 76%ee
- 20 Chiral analytical HPLC (Chiralpak AD column, 4.6 x 250mm, eluent 50:50:0.1 MeOH: EtOH: Butylamine, flow rate 0.5ml/min, UV detection at 220nm), Rt 8.9min.

Description 7: 2-[4-(3,4-Dichloro-benzyl)-morpholin-2-ylmethyl]-isoindole-1,3-dione

- 25 dione
- A mixture of 2-[(3,4-dichlorobenzyl)amino]ethanol (2.038 g) and (S)-2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione (2.032g) in tetrahydrofuran (3.3ml) was stirred and heated at reflux under nitrogen. After 21.5h more tetrahydrofuran (12.5ml) was added and the mixture was cooled to 3°. Triphenyl phosphine
- 30 (2.793g) was added and the mixture was stirred until all the solid had dissolved. Diisopropylazodicarboxylate (2.1ml) was then added over 12min maintaining the temperature at <7°. After 2.25h the mixture was allowed to warm to 22°. After 5.3h more triphenylphosphine (121mg) and diisopropylazodicarboxylate (0.09ml) were added. After 22.5h the reaction mixture was concentrated to near dryness.
- 35 Propan-2-ol (12ml) was added and the concentration repeated, this was repeated once more. More propan-2-ol (12ml) was added and the mixture was heated to 70°. After 0.5h the slurry was cooled to 22° and then after a further 2h the product was collected by filtration. The bed was washed with propan-2-ol (2x4ml) and then dried *in vacuo* at 40° to give the product, (2.622g).

Description 8: 4-Nitrophenyl [(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methylcarbamate

To a solution of 4-nitrophenyl chloroformate (2.92g) in dry dichloromethane (10ml) at 0° under nitrogen was added solution of Description 5 (4.0g) and triethylamine (2.07ml) in dry dichloromethane (15ml) drop-wise over 10 min. The mixture was allowed to warm to room temperature and stirred for 2.5h. The solvent was evaporated and the residue purified by Biotage flash chromatography on silica gel, eluting with 25-50% ethyl acetate in cyclohexane, to give the title compound (3.8g).

10 LC-MS (System A) Rt 3.1mins. Mass Spectrum m/z 440 [MH⁺].

Description 9: Methyl 3-([(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl)amino)carbonyl]amino)methyl)benzoate

- To a stirred solution of 3-aminomethyl-benzoic acid methyl ester (0.1817g) (Eur. J. Med. Chem. 1993, 28(7-8), 624-632) in N,N-dimethylformamide (4ml) was added a solution of Description 8 (0.4612g) in N,N-dimethylformamide (4ml). N,N-diisopropylethylamine (0.365ml) was added, and the mixture was stirred at 20°C for 4h. After leaving to stand for a further 67h, the mixture was diluted with methanol (ca. 5ml) and applied to 2x10g SCX ion-exchange cartridges (IST Isolute Flash SCX-2), pre-conditioned with methanol. The cartridges were eluted with methanol and 10% 0.880 ammonia solution in methanol, and the first ammonia fractions combined and the solvent evaporated *in vacuo*. The residue was purified by Biotage™ flash chromatography on silica gel, eluting with 200:8:1 dichloromethane/ethanol/0.880 ammonia solution, to give the title compound (0.4237g) as a pale yellow gum.

LC/MS (System A): R_t = 2.59min, m/z 466,468 [MH⁺]

Description 10: 3-([(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl)amino)carbonyl]amino)methyl)benzoic acid

- To a stirred solution of Description 9 (0.3869g) in methanol (10ml) and warm propan-2-ol (7ml) was added 2M aqueous sodium hydroxide (0.85ml) and water (5ml). The mixture was stirred at 20°C for 20h before the solvents were evaporated *in vacuo* to leave a white solid. The solid was re-dissolved in water and the pH of the solution was adjusted to approximately 6 by the addition of 2M aqueous hydrochloric acid. The mixture was applied to 2x10g SCX ion-exchange cartridges (IST Isolute Flash SCX-2), pre-conditioned with methanol. The cartridges were eluted with methanol and 5% triethylamine in methanol. The product was found to have eluted in the first methanol fractions which were combined and the solvent evaporated *in vacuo* to recover the crude product.
- The solid was re-dissolved in water and the pH of the solution adjusted to

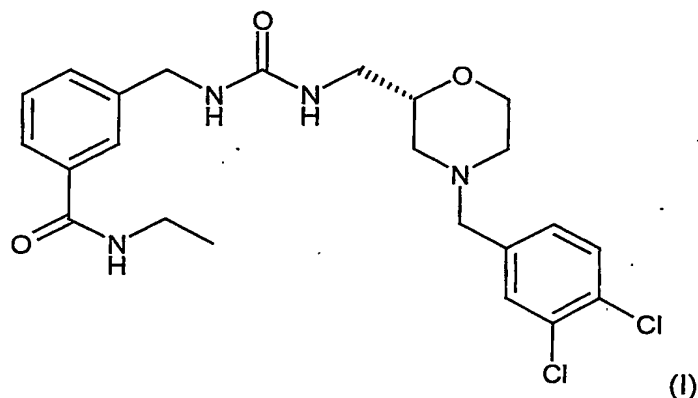
- approximately 1 by the addition of 2M aqueous hydrochloric acid. The mixture was applied to 2x10g SCX ion-exchange cartridges (IST Isolute Flash SCX-2), pre-conditioned with methanol. The cartridges were eluted with methanol and 5% triethylamine in methanol. The first triethylamine fractions were combined and the solvent was evaporated *in vacuo* to give the title compound (0.186g) as a light brown oil.
- LC/MS (System A): $R_t = 2.45\text{min}$, m/z 452,454 $[\text{MH}^+]$

10 Example

- 3-([[(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl]amino)carbonyl]-amino)methyl)-N-ethylbenzamide
- To a stirred solution of Description 10 (0.035g) in N,N-dimethylformamide (1ml) was added a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.0208g) and 1-hydroxybenzotriazole (0.012g) in N,N-dimethylformamide (1ml) at 20°. N,N-diisopropylethylamine (0.0196ml) and a 2M solution of ethylamine in tetrahydrofuran (0.282ml) were added, and the mixture was stirred at 20°C for 18.5h. The mixture was diluted with methanol (ca. 2ml) and applied to a 5g SCX ion-exchange cartridge (IST Isolute Flash SCX-2), pre-conditioned with methanol. The cartridge was eluted with methanol and 10% 0.880 ammonia solution in methanol. The first ammonia fraction was evaporated *in vacuo* and the residue further purified by Biotage™ flash chromatography on silica gel, eluting with 100:8:1 dichloromethane/ethanol/0.880 ammonia solution, to give the title compound (0.0107g) as a colourless glass.
- LC/MS (System A): $R_t = 2.35\text{min}$, m/z 479,481 $[\text{MH}^+]$

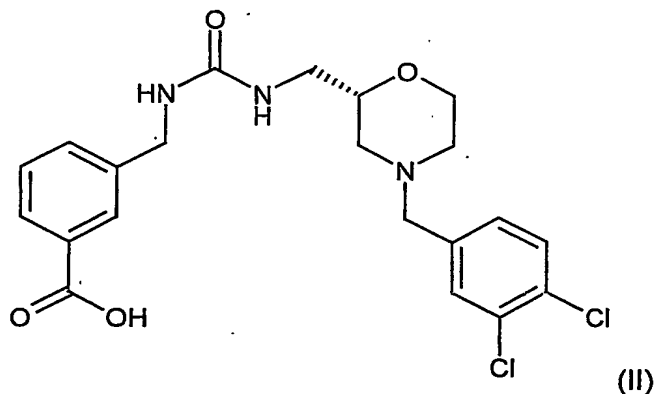
Claims

- 5 1. A compound of formula (I):



and salts and solvates thereof.

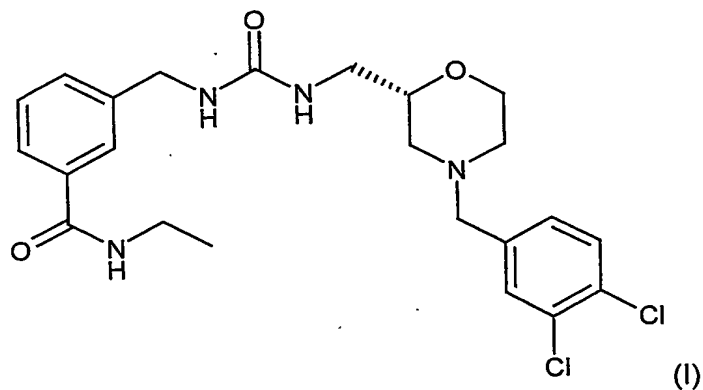
- 10 2. A process for the preparation of a compound of formula (I) or a salt or solvate thereof which process comprises coupling of a compound of formula (II) or a salt thereof with ethylamine



- 15 3. A compound as claimed in claim (I) for use as a therapeutic agent.
4. A method of treatment of a human or animal subject suffering from or susceptible to an inflammatory condition which method comprises
- 20 administering an effective amount of a compound as claimed in claim 1.
5. A pharmaceutical composition comprising a compound as claimed in claim 1 and a physiologically acceptable carrier therefor.

Abstract

Compound of formula (I):



5

which is;

3-({[[(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl]amino}carbonyl]-
-amino)methyl)-N-ethylbenzamide;

10 and salts and solvates thereof, a process for its preparation, pharmaceutical
formulations containing it and its use in therapy.

PCT Application
PCT/EP2003/003338

